Neural Stem Cells

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This article is concerned with the idea that neural precursor cells in vertebrates can self-renew and give rise to all cell types within the nervous system. Supportive evidence for this notion of neural stem cells comes from clonal analyses undertaken both *in vivo* and *in vitro*. Neural stem cells also give rise to other cells in the body, including skin melanocytes and a range of mesenchymal cells in the head and neck. What determines the fate of these stem cells is their initial location within the developing neural tube and their final location post migration from the proliferative zone of the neural tube. A population of cells in the adult brain also have the characteristics of classical stem cells, a finding that opens the way for potential replacement therapy in nervous system–degenerative diseases. Much of the work in our laboratory has been concerned with the regulation of expansion and differentiation of these cells into their myriad progeny and the role of a series of various growth factors in this process. Different factors, such as members of the fibroblast growth factor family, act at different times to regulate stem cell proliferation and differentiation. Some factors, including members of the TGFβ superfamily, appear to be directly involved in the specification of cell fate. Finally, we are beginning to be able to determine the steps in the development of some lineages from multipotential stem cell to fully functional differentiated cell. ***Key words: neural crest/fibroblast growth factor/leukemia inhibitory factor/Steel factor/endothelin/melanocytes/neuronal differentiation. Journal of Investigative Dermatology Symposium Proceedings 2:8–13, 1997***

The idea of a stem cell, from which all cell types in a particular organ or system in the body are derived, gained most support historically from work in the field of development of the blood. Studies of blood development show that some cells have the capacity to both self-renew and to give rise to all known blood cell types (Metcalf, 1989). These are defined as blood stem cells. The primary progeny of these stem cells are other stem cells and, additionally, a more restricted class of undifferentiated cell, a progenitor cell. The progenitor cell can also self-renew, but it can only differentiate into one or more subclasses of cell. Thus, there is a developmental hierarchy of cells, from the multipotential stem cell through progressive stages of restriction until, finally, a cell whose fate is restricted to a single lineage.

This kind of developmental hierarchy has also been proposed in the development of the nervous system. and there is now strong evidence that such a system operates. There are two major differences, however, between the development of blood and development of the nervous system: first, blood cells continue to be generated throughout the life of the animal whereas the nervous system is effectively generated only during embryogenesis. Second, whereas blood develops and operates without many spatial cues, the nervous system develops initially within the context of a tube of cells, the neural tube. The location of any particular cell within that tube defines its fate both along an anterior-posterior axis as well as dorso-ventrally. Additionally, the neurons form massive three-dimensional connections with each other, which more or less define the final function of the brain and spinal cord. These differences must clearly influence the way in which the stem cells of the nervous system have their fates specified. Thus, because the nervous system develops in a very defined spatial and temporal context, the local environment around any particular cell might be discrete, and thus the fate of any given stem cell within the nervous system might be determined by the local environment.

Are the Cells That Give Rise to the Nervous System Genuine Stem Cells? The emerging evidence is that the nervous system *is* derived from multipotential stem cells. The full potentiality of every stem cell, however, may not be realized *in vivo*; i.e., not every stem cell gives rise to all possible derivatives *in vivo*, and the probable fate of most stem cells is a smaller range of derivatives than their full potential.

Clonal Analysis *In Vivo* How has this view of nervous system development emerged? The primary question of whether genuine stem cells exist in the developing brain has generally been addressed by determining whether single cells within the different parts of the developing nervous system can give rise to the two major classes of neural cells, neurons and glia. These studies have been undertaken both *in vivo* and *in vitro*. The *in vivo* studies involved the tagging of cells within the neural tube by either infection with retroviruses carrying the lac Z gene or by direct injection of fluorescent dyes into individual neural tube cells and then following the progeny of these cells. Studies using retrovirus infection have come mainly from the laboratories of Cepko and Sanes and provide evidence for precursors in the brain that give rise not only to multiple types of differentiated progeny, but also to precursors that produce a much more restricted range of cell types (Gray *et al*, 1990; Sanes, 1994). Precursors are a general term for cells that have the potential to give rise to more differentiated cells and thus include stem cells and progenitor cells. When a highly complex retroviral library was used

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Abbreviations: LIF, leukemia-inhibitory factor; CNTF, ciliary neurotrophic factor.

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to infect the diencephalon, which gives rise to the thalamus, hypothalamus, and epithalamus, retrovirally marked clones were found to contain neurons, glia, and occasionally radial glia (Golden and Cepko, 1996). The majority of clones dispersed in all directions and included clones populating multiple nuclei within the diencephalon and even clones that crossed major boundaries of the developing nervous system, such as the diencephalon and mesencephalon, which give rise to completely different brain structures. These findings showed that precursor cells in the diencephalon are multipotent and that their daughter cells can become widely dispersed (Golden and Cepko, 1996).

Likewise, studies with direct injection of fluorescent-labeled dextrans in different parts of the nervous system, such as the frog neural retina, show that individual precursors are multipotent and are capable of producing many combinations of cell types (Wetts and Fraser, 1991).

This approach has also been used extensively in the analysis of the neural crest, whose cells form part of the developing neural tube, but which migrate away from the neural tube and into the rest of the embryo to give rise to a vast array of derivatives including the sensory, autonomic, and enteric ganglia of the peripheral nervous system, the melanocytes of the skin, and a large range of tissue types in the head and upper body, including bone, muscle, and cartilaginous tissue. Single neural crest cells have been micro injected with a fluorescent dye prior to migration from the neural tube (Bronner-Fraser and Fraser, 1989; Bronner-Fraser *et al*, 1991; Fraser and Bronner-Fraser, 1991). The clonal progeny of the cells, after 2 d, were sometimes found to be distributed in many regions to which neural crest cells normally migrate. Although the phenotype of these cells could not be definitively ascribed, it was found, on the basis of morphology and antibody binding, that individual clones contained sensory neurons, presumptive melanoblasts, satellite cells in dorsal root ganglia, adrenomedullary cells, and neural tube cells. Thus, these findings support the existence of multipotential neural crest cells *in vivo*. Some cells in the neural tube also appeared to give rise to both neural crest cells and neural tube cells destined to become mature central nervous system cells (Bronner-Fraser and Fraser, 1989; Bronner-Fraser *et al*, 1991). Although these studies indicate the diversity of cell products, there has been concern as to whether they represent the progeny of a single cell. Difficulties with confining the injection to only one cell appears to present considerable technical difficulties to other workers. Results obtained by following the progeny of neural crest cells infected with lac-Z containing retrovirus *in vivo* in the dorsal root ganglia, however (Frank and Sanes, 1991), also support the multipotential concept for the neural crest.

Clonal Analysis *In Vitro* The other approach to studying the potential of neural precursor cells is to examine the potential of the precursor cells to develop *in vitro*. The *in vitro* approach allows one to examine what the full potential of any particular cell might be, and thus can give quite a different answer to *in vivo* analyses. The difference between this approach and the *in vivo* approach is that the analysis of cell lineage *in vivo* explains what actually happens to the cells within the particular preset environment of the developing animal. Using an *in vitro* analysis, it was first shown by Temple (1989) that septal precursor cells were both heterogeneous in potentiality, but that approximately one quarter of the clones generated were multipotential. Work from our laboratory essentially confirmed this finding but, in addition, showed that the precursor cells were classical stem cells in that they had a very large capacity for self-renewal (Kilpatrick and Bartlett, 1993).

Clonal analyses have also been undertaken in studies of the neural crest. Sieber-Blum and Cohen (1980) first used clonal analysis to study quail neural crest cells and found a proportion of clones that contained both catecholaminergic (neuronal lineage) and pigmented cells. More recent studies (Duff *et al*, 1991; Ito and Sieber-Blum, 1991) have revealed different classes of clones: clones exclusively of the melanogenic lineage, clones that were unpigmented, and clones containing both pigmented and unpigmented cells (mixed). The unpigmented and mixed clones all contained both catecholaminergic and sensory neurons. Thus, in this system, there is evidence for tripotent cells, cells restricted to two lineages, and fully committed cells. In the latter study (Ito and Sieber-Blum, 1991), a clonal analysis of the cardiac neural crest, pluripotential (mesenchymal, neuronal, and melanocytic), bipotential (mesenchymal, neuronal), and fully restricted clones were found.

Studies from the laboratory of Le Douarin found evidence for a similarly heterogeneous range of clones (Baroffio *et al*, 1988; Dupin *et al*, 1990; Baroffio *et al*, 1991). In these studies, in addition to the fully restricted clones, multipotent clones comprising neurons, pigmented cells, and nonneuronal cells were found, as well as more restricted clones that contained Schwann cells, satellite cells, and neurons, but not pigmented cells. This pattern may indicate that neural and melanocytic cell precursors segregate early in the differentiation process. In one study (Baroffio *et al*, 1991), some multipotential clones were found to contain either the full array of neural crest derivatives, including mesenchymal elements, or were restricted to either neural and melanocytic or neural and mesenchymal.

These studies support the idea that within any neural crest population there are both multipotential and committed cells at the migratory stage. The observation of considerable heterogeneity in the clones is not necessarily an indication that there is innate heterogeneity in the neural crest cell's repertoire. It may be that all neural crest cells are initially multipotent; however, at the time the cells are isolated they have reached different stages of differentiation. If this is the case, then the actual lineage pathways, or committment steps, may be inferred from the segregation pattern observed in the clones. Within the multipotent clones, some are either neural/melanocytic or neural/mesenchymal, which suggests that this may be the first restrictive choice the neural crest cells make. Neurons, melanocytes, and mesenchymal cells segregate from each other in the more restricted clones. Glial cells also segregate from the other cell types, but a significant number of clones show co-segregation of neurons and glial cells, indicating the existence of a common glial/neuronal precursor that retains its bipotentiality late into the differentiation process. These findings, especially with the many intermediate, or partially committed clones, supports the concept of sequential differentiation from a multipotential cell. This concept is represented diagrammatically in **Fig 1**.

An alternative view of these studies is that the heterogeneity of clones that have been found in the neural crest is actually a random assortment of clonal progeny and that many of the cells are fully multipotent. Their clonal progeny do not contain all possible neural crest derivatives because the differentiation process *in vitro* is simply randomized; all combinations of derivatives might be expected. The reality is probably somewhere in between these two views. In other words, there is probably some progressive restriction of cell fate from a truly pluripotential cell through more restricted cell types, but the major sublineages are specified relatively early, and this specification is controlled by particular growth factors (see below).

Stem Cells in the Adult Brain That neurogenesis is complete by the time an animal is born and that the nervous system has a severely restricted regenerative capacity in mammals probably represent good reasons to believe that there are no cells in the brains of adult mammals that have the characteristics of neural stem cells. This is not the case in fish, amphibians, and even some birds, however, because cells in the subventricular zone of these animals retain the capacity to proliferate and differentiate into neurons (Alvarez-Buylla *et al*, 1990). In birds, it was originally thought that these subventricular cells specifically populated higher vocal centers in the forebrain in response to hormonal stimuli (Nordeen and Nordeen, 1990); however, more recent studies indicate that there is actually quite a large turnover in avian brains and that much of the forebrain is turned over (A. Doupe, personal communication).

This is clearly not the case in the mammalian brain but it does

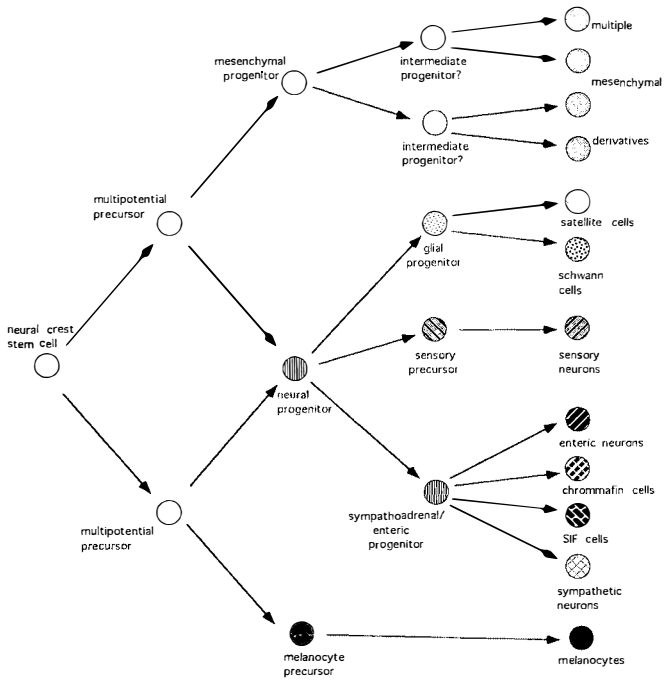

Figure 1. Cell lineage map of the neural crest. The construction of this map is based on studies of single cell cloning experiments and characterization of antigen expression patterns as outlined in the text. The full complement of neural crest derivatives is normally only seen in the rostral regions of the neural crest.

appear that there are cells within the adult mammalian brain with the properties of neural stem cells. Reynolds and Weiss (1992) isolated cells from the adult mouse striatum, induced the proliferation of precursors within the cultures by administering epidermal growth factor, and found that the precursors differentiated into neurons and glia when the epidermal growth factor was withdrawn. Studies in our laboratory (Richards *et al*, 1992b) show that precursors could be induced to differentiate into neuronal cells when they were initially stimulated *in vitro* with fibroblast growth factor-2 (FGF2) and then with medium conditioned by an astrocyte cell line. The multipotential nature of these cells has been shown by clonal analysis, with such clones being able to give rise to both neurons and glia. The role of such cells, in rodents at least, might be to migrate to the olfactory bulb and differentiate into interneurons (Luskin, 1993; Lois *et al*, 1996).

Whether such cells exist in humans is at present unclear, although it has been reported that neuron-like cells can be generated *in vitro* from human temporal lobe periventricular tissue.[1] If such cells do exist in humans, it opens the way for potential replacement therapy in central nervous system diseases. Although this may seem a fanciful notion at present because creating functional connections within a diseased or damaged brain appears to be extremely difficult, some early experiments, nevertheless, are promising. For example, when neural stem cells were genetically engineered to carry a therapeutic enzyme (β-glucuronidase) and transplanted, they corrected a mouse model of Sly disease (Snyder *et al*, 1995). In addition, work from Gage's laboratory shows that it may be possible to stimulate axon regrowth in an injured brain with appropriate application of growth factors (Aubert *et al*, 1995).

Growth Factor Regulation of Neural Stem Cells There is now increasing evidence that soluble growth factors are at least partially responsible for the regulation of the expansion of the neural stem cell pool in the embryo as well as in the control of the development of the nervous system generally. Our laboratory has been extensively involved in determining the regulation of expansion of this stem cell pool in which factors regulate the differentiation of these stem cells into all of the components of the nervous system as well as other neural crest derivatives.

Regulation of Stem Cell Survival and Proliferation Epigenetic factors may act upon neural stem cells either to stimulate their survival, to induce their proliferation, or to facilitate their specification or differentiation into mature cell phenotypes. We have found that insulin-like growth factor 1 is a necessary epigenetic requirement for the survival of stem cells isolated from E10 murine telencephalon and mesencephalon (Drago *et al*, 1991). In this system, it appears that the insulin-like growth factor 1 is produced by the stem cells themselves, and thus regulation of survival appears to be an autocrine function.

In addition, we have found that members of the fibroblast growth factor (FGF) family induce the proliferation of central nervous system stem cells (Murphy *et al*, 1990). At high cell density, FGF also induces the differentiation of these stem cells into neurons and astrocytes (Murphy *et al*, 1990). This latter response, however, may have resulted from the secondary production of other factors within the cultures. Clonal studies provide a more precise means of analyzing these potential influences. Using clonal analysis, we found that FGF alone was unable to induce the differentiation of these stem cells (Kilpatrick and Bartlett, 1993), and thus its primary activity at this stage of development is proliferation. This singular activity of FGF, however, may only occur when the stem cells are within the ventricular region of the neural tube.

We have also shown that most, if not all, neural crest cells are stimulated to divide in the presence of FGF, and thus it almost definitely stimulates neural crest stem cell division, although for these cells, factors in serum are also required (Murphy *et al*, 1994). In addition, stem cells from the embryonic spinal cord proliferate in response to FGF (M. Murphy, unpublished data), and Ray and Gage (1994) showed that FGF2 stimulates spinal cord neuronal precursor proliferation. Thus, FGF2 stimulates proliferation of stem cells for the entire nervous system. FGF2 is sequestered in the basement membrane around the neural tube (Kalcheim and Neufeld, 1990), and in association with a heparan sulfate proteoglycan within the neuroepithelium (Ford *et al*, 1994). Our *in situ* hybridization analysis shows specific expression of FGF2 within the neural tube and developing dorsal root ganglia at embryonic day 10 (Murphy *et al*, 1994). The cells in the neural tube that are positive for FGF2 mRNA are predominantly proliferating cells. Likewise, this corresponds to a time when most of these dorsal root ganglia cells are proliferating (Lawson *et al*, 1974), suggesting autocrine regulation of proliferation within these stem cells. FGF2 activity may also be regulated through specific interaction with heparan sulfate proteoglycan (Nurcombe *et al*, 1993; Ford *et al*, 1994).

Epidermal growth factor may also be involved in the stimulation of neural precursor cells (Anchan *et al*, 1991; Reynolds *et al*, 1992); however, we have found that its activities may be restricted to glial progenitors and that it does not stimulate stem cell proliferation (Kilpatrick and Bartlett, 1995).

Factors That Regulate Stem Cell Differentiation One would expect, given the vast array of cell types that exist in the nervous system as well as other cell types derived from the neural crest, that there would also be many factors that are involved in the regulation of phenotype of these cells. This is probably the case; however, although a number of growth factors have been implicated in this process, there are still many steps in the development of these cells that have not been defined. I will address some of the known pathways in differentiation of neural stem cells and, in particular, those pathways of interest to our laboratory.

FGF FGF may not only stimulate proliferation of stem cells but under different conditions, members of the FGF family may stimulate neuronal differentiation. For example, FGF1 and FGF2 may

[1] Kirshenbaum B, Nedergaard M, Preeuss A, Bahramian K, Goldman SA: *In vitro* neuronal and glial production and differentiation by precursor cells derived from the adult human brain. *Soc Neurosci Abstr* 704:3, 1993 (abstr).

have different roles in early neural development. The expression patterns of these molecules show that FGF2 is expressed at the time of precursor proliferation (E10) but that FGF1 is first expressed at E12, corresponding to the time of neurogenesis. These factors are presented to the FGF receptors in conjunction with specific heparan sulfate proteoglycans, and it appears that precursor proliferation or differentiation may be favored by different combinations of FGFs and proteoglycan side chains (Nurcombe *et al*, 1993).

In addition, as these cells move away from the ventricular region of the neural tube, their response to FGF may change significantly. If cells are taken from older embryos and treated with FGF in clonal cultures, some of the resulting clones express neuronal markers and have the morphology of immature neurons (Kilpatrick and Bartlett, 1995). In the case of neural crest cells, when these cells are emerging from the neural tube and in the process of migration, their primary response to FGF is proliferation (Murphy *et al*, 1994). Some of these cells form the developing sensory ganglia where they continue to proliferate for a short time and then differentiate. If these sensory ganglia cells are taken from the embryo immediately after ending their proliferation *in vivo* (E12 in mice), they do not respond to FGF by proliferating but by differentiating into sensory neurons (M. Murphy, unpublished observations). Thus, it is possible that as the cells change, their FGF receptor expression changes, and they respond to different combinations of FGFs and heparan sulfate proteoglycans and differentiate.

Neurotrophic Cytokines Neurotrophic cytokines are a group of structurally related factors that include leukemia-inhibitory factor (LIF) ciliary neurotrophic factor (CNTF), oncostatin M, growth-promoting activity, and interleukin 6 (Bazan, 1991). These factors also have similar signaling pathways and act through receptor complexes containing shared components (Gearing *et al*, 1992; Ip *et al*, 1992). The first characterized member of the group, LIF, was originally described almost 20 y ago as a factor that regulates transmitter phenotype in neurons (Patterson, 1978). We found that LIF stimulated neuronal generation of the stem cells of the peripheral nervous system, the neural crest (Murphy *et al*, 1991). These neurons have sensory characteristics, and we later found that LIF also stimulates sensory neuron differentiation of precursor cells in developing sensory ganglia (Murphy *et al*, 1993). In addition, neuronal differentiation of spinal cord stem cells is dependent on factors acting through the LIF receptor (Richards *et al*, 1992b). In later experiments we showed that LIF acts directly on neuronal precursors and is primarily a differentiation factor (Richards *et al*, 1996).

These cytokines also have been implicated in glial development. CNTF, together with serum and extracellular matrix, induces the *in vitro* differentiation of type 2 astrocytes from O-2A progenitors isolated from rat optic nerve (Hughes *et al*, 1988; Lillien and Raff, 1990). Recently both CNTF and LIF have been shown to promote the differentiation of glial progenitors, derived from the optic nerve, into both oligodendrocytes and astrocytes (Mayer *et al*, 1994). We found that in the presence of serum, LIF potentiates the differentiation of stem cells into GFAP-positive astrocytes (Richards *et al*, 1996). Together, these results suggest that LIF, or related cytokines, act as differentiation agents for spinal cord stem cells, regardless of their lineage. Thus the action of these molecules is not to specify cell lineage, but rather to potentiate the differentiation process.

So far, mice deleted for either *LIF* (Stewart *et al*, 1992) or *CNTF* genes (Masu *et al*, 1993) have revealed no defects in the development of spinal cord neurons. Recent observations, however, reveal developmental defects in spinal cord neurons and astrocytes of mice deleted for a component of the LIF receptor (Ware *et al*, 1995; S. Koblar, M. Murphy, P.F. Bartlett, unpublished observations). These findings suggest that factors apart from LIF or CNTF, but that signal through LIF receptors, are involved in the differentiation process.

Lineage Specification Factors One of the big unanswered questions in development is how the fate of stem cells is decided. In blood development there is no good evidence that stem cell fate is specified; rather it is proposed to be a stochastic process, whereby a stem cell will differentiate down one lineage or the other randomly. The newly differentiated cells then become dependent on lineage-specific growth factors for their survival and proliferation, and thus different cell types are selected for rather than being specified by growth factors. Recent information suggests that this may not be the case in the nervous system and that there may well be lineage-specifying factors in addition to lineage selection factors. Studies in rat neural crest show that glial growth factor, previously defined as a Schwann cell mitogen, strongly suppresses neuronal differentiation of the stem cells while promoting or allowing glial differentiation (Shah *et al*, 1994). Clonal analysis suggests that the glial growth factor is specifying cell fate rather than being selective (Shah *et al*, 1994).

We have found that stem cells from the spinal cord specifically give rise to motor neuron-like cells when plated onto an astrocyte precursor cell line (Richards *et al*, 1995). Time-lapse analysis revealed that the great majority of precursors can be induced to differentiate into the motor neuron phenotype by factors produced by Ast-1 cells, suggesting that a similar factor(s) produced by cells

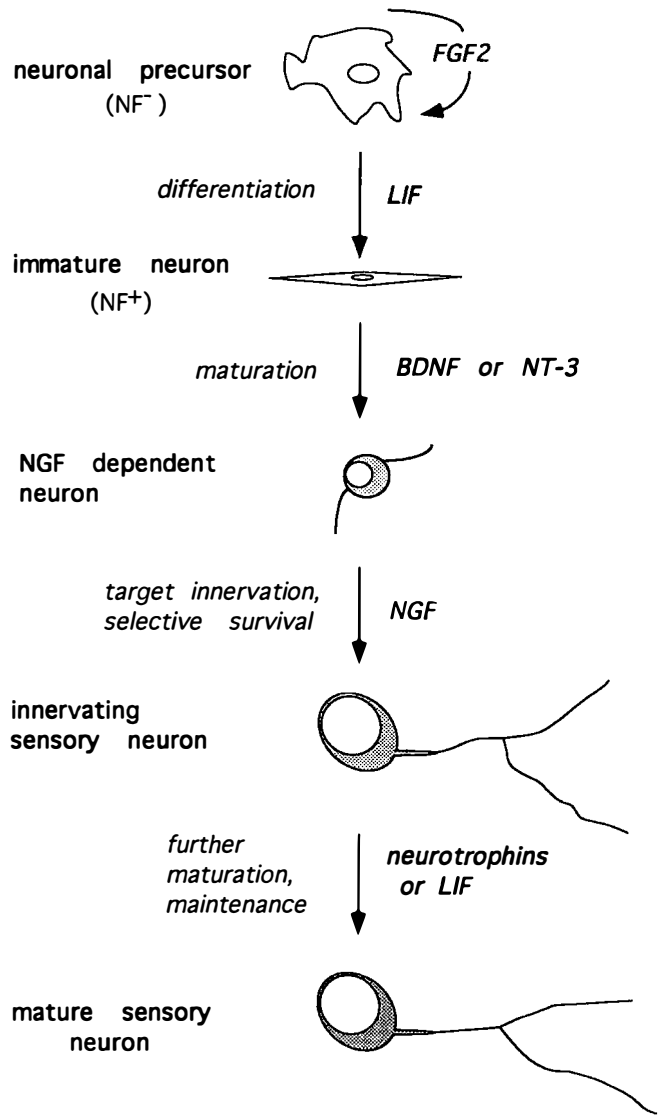

Figure 2. A scheme for the development of sensory neurons from the neural crest. In this scheme, the development of sensory neurons is seen as a series of steps, each of which is controlled or promoted by a particular factor or factors.

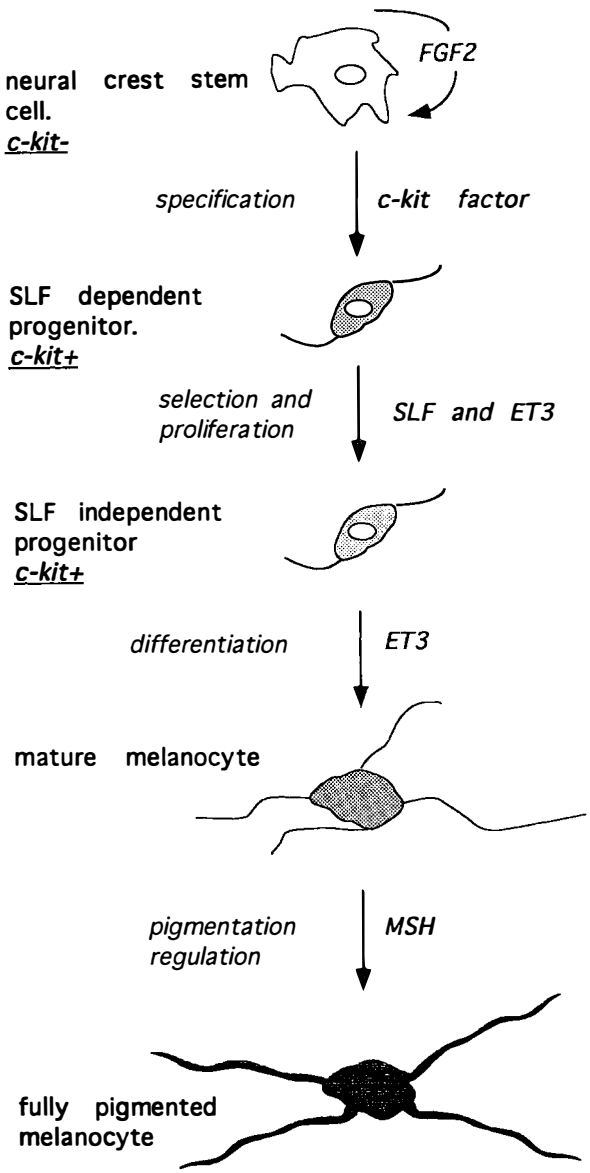

Figure 3. Summary scheme of the roles of different growth factors in melanocyte development.

akin to Ast-1 may specify the motor neuron lineage *in vivo*. A candidate factor responsible for motor neuron specification is sonic hedgehog, which induces expression of a transcription factor, Isl-1, found in motor neurons (Pownall, 1994). We have found, however, that sonic hedgehog alone is insufficient to induce motor neuron differentiation (R. Dutton and M. Murphy, unpublished observations).

Recently, a member of the transforming growth factor-β (TGF-β) family, bone morphogenic protein 2, was found to rapidly induce the production of a neuronal-specific transcription factor, MASH1, in neural crest stem cells (Shah *et al*, 1996). The MASH1$^+$ cells then differentiated into neurons. *In vivo*, MASH1$^+$ cells are located near sites of bone morphogenic protein 2 mRNA expression. Another member of the TGF-β family, TGF-β1, exclusively promotes smooth muscle differentiation (Shah *et al*, 1996). Thus, alternative neural crest cell fates are specified by different TGF-β family members, and these data further support the idea that neural stem cells are directly specified into particular lineage pathways.

Toward the Definition of Pathways from Stem Cell to Terminal Differentiation Our studies with LIF on sensory neurons were extended to examine the role of other growth factors in sensory development. We found that as the sensory neurons developed, different growth factors came into play. These studies could be integrated into a pathway of sensory development, wherein each step in the pathway represents a different stage in the development of the sensory lineage and is regulated by at least one growth factor. Such a pathway is shown in **Fig 2**.

Neural stem cells give rise not only to the nervous system, but also to other cell types in the body including skin melanocytes. Because melanocytes regulate skin and hair color, it has been relatively easy to isolate murine mutants that have coat color variation and alterations in melanocytes or in melanocyte development. Some of these mutants have mutations in genes for growth factors or receptors, indicating that such growth factor signaling is required for proper melanocyte development and function. We have used this information and studied the functions of these growth factors in melanocyte development (Murphy *et al*, 1992; Reid *et al*, 1995). We have incorporated these findings into a pathway of melanocyte development **(Fig 3)**. The receptor for one of the factors involved, Steel factor, is exclusively expressed by melanocyte progenitor cells within the neural crest population, and the subsequent survival and proliferation of these cells are regulated by Steel factor. Thus, this factor is acting as a classic selective factor. Another factor implicated in this pathway is endothelin 3, which also acts on the melanocyte progenitors to correlate their survival and proliferation with Steel factor (Reid *et al*, 1996). In addition, endothelin 3 stimulates the differentiation of these cells into mature pigmented melanocytes (Reid *et al*, 1996). The classic melanocyte factor, melanocyte stimulating hormone, can then act on these mature melanocytes to regulate the degree of their pigmentation. Finally, we have discovered an as yet uncharacterized factor that has the ability to upregulate the steel factor receptor on neural crest stem cells (c-*kit* factor), and thus this factor may be involved in the specification of the melanocyte lineage directly from the neural crest stem cells (K. Reid and M. Murphy, unpublished data).

REFERENCES

Alvarez-Buylla A, Kirn JR, Nottebohm F: Birth of projection neurons in adult avian brain may be related to perceptual or motor learning. *Science* 249:1444–1446, 1990

Anchan RM, Reh TA, Angello J, Balliet A, Walker M:. EGF and TGF-alpha stimulate retinal neuroepithelial cell proliferation *in vitro*. *Neuron* 6:923–936, 1991

Aubert I, Ridet JL, Gage FH: Regeneration in the adult mammalian CNS: guided by development. *Curr Opin Neurobiol* 5:625–635, 1995

Baroffio A, Dupin E, Le DN: Clone-forming ability and differentiation potential of migratory neural crest cells. *Proc Natl Acad Sci USA* 85:5325–5329, 1988

Baroffio A, Dupin E, Le DN: Common precursors for neural and mesectodermal derivatives in the cephalic neural crest. *Development* 112:301–305, 1991

Bazan JF: Neuropoietic cytokines in the hematopoietic fold. *Neuron* 7:197–208, 1991

Bronner-Fraser M, Fraser S: Developmental potential of avian trunk neural crest cells *in situ*. *Neuron* 3:755–766, 1989

Bronner-Fraser M, Stern CD, Fraser S: Analysis of neural crest cell lineage and migration. *J Craniofac Genet Dev Biol* 11:214–222, 1991

Drago J, Murphy M, Carroll SM, Harvey RP, Bartlett PF: Fibroblast growth factor-mediated proliferation of central nervous system precursors depends on endogenous production of insulin-like growth factor I. *Proc Natl Acad Sci USA* 88:2199–203, 1991

Duff RS, Langtimm CJ, Richardson MK, Sieber-Blum M: *In vitro* clonal analysis of progenitor cell patterns in dorsal root and sympathetic ganglia of the quail embryo. *Dev Biol* 147:451–459, 1991

Dupin E, Baroffio A, Dulac C, Cameron CP, Le Douarin N: Schwann-cell differentiation in clonal cultures of the neural crest, as evidenced by the anti-Schwann cell myelin protein monoclonal antibody. *Proc Natl Acad Sci USA* 87:1119–1123, 1990

Ford MD, Bartlett PF, Nurcombe V: Co-localization of FGF-2 and a novel heparan sulphate proteoglycan in embryonic mouse brain. *Neuroreport* 5:565–568, 1994

Frank E, Sanes JR: Lineage of neurons and glia in chick dorsal root ganglia: analysis *in vivo* with a recombinant retrovirus. *Development* 111:895–908, 1991

Fraser SE, Bronner-Fraser M: Migrating neural crest cells in the trunk of the avian embryo are multipotent. *Development* 112:913–920, 1991

Gearing DP, Comeau MR, Friend DJ, Gimpel SD, Thut CJ, McGourty J, Brasher, KK, King JA, Gillis S, Mosley B, Zeigler SF, Cosman D: The IL-6 signal transducer, gp130: an oncostatin M receptor and affinity converter for the LIF receptor. *Science* 255:1434–1437, 1992

Golden JA, Cepko CL: Clones in the chick diencephalon contain multiple cell types and siblings are widely dispersed. *Development* 122:65–78, 1996

Gray GE, Leber SM, Sanes JR: Migratory patterns of clonally related cells in the developing central nervous system. *Experientia* 46:929–940, 1990

Hughes SM, Lillien LE, Raff MC, Rohrer H, Sendtner M: Ciliary neurotrophic factor induces type-2 astrocyte differentiation in culture. *Nature* 335:70–73, 1988

Ip NY, Nye SH, Boulton TG, Davis S, Taga T, Li Y, Birren SJ, Yasakawa K, Kishimoto T, Anderson DJ, Stahl N, Yancopoulos GD: CNTF and LIF act on neuronal cells via shared signaling pathways that involve the IL-6 signal transducing receptor component gp-130. *Cell* 69:1121–1132, 1992

Ito K, Sieber-Blum M: *In vitro* clonal analysis of quail cardiac neural crest development. *Dev Biol* 148:95–106, 1991

Kalcheim C, Neufeld G: Expression of basic fibroblast growth factor in the nervous system of early avian embryos. *Development* 109:203–215, 1990

Kilpatrick TJ, Bartlett PF: Cloning and growth of multipotential neural precursors: requirements for proliferation and differentiation. *Neuron* 10:255–265, 1993

Kilpatrick TJ, Bartlett PF: Cloned multipotential precursors from the mouse cerebrum require FGF-2, whereas glial restricted precursors are stimulated with either FGF-2 or EGF. *J Neurosci* 15:3653–3661, 1995

Lawson SN, Caddy KWT, Biscoe TJ: Development of rat dorsal root ganglion neurones. Studies of cell birthdays and changes in mean cell diameter. *Cell Tissue Res* 153:399–403, 1974

Lillien LE, Raff MC: Differentiation signals in the CNS: type-2 astrocyte development *in vitro* as a model system. *Neuron* 5:111–119, 1990

Lois C, Garcia-Verdugo JM, Alvarez-Buylla A: Chain migration of neuronal precursors. *Science* 271:978–981, 1996

Luskin MB: Restricted proliferation and migration of postnatally generated neurons derived from the forebrain subventricular zone. *Neuron* 11:173–189, 1993

Masu Y, Wolf E, Holtman B, Sendtner M, Brem G, Thoenen H: Disruption of the CNTF gene results in motor neuron degeneration. *Nature* 365:27–32, 1993

Mayer M, Bhakoo K, Noble M: Ciliary neurotrophic factor and leukemia inhibitory factor promote the generation, maturation and survival of oligodendrocytes *in vitro*. *Development* 120:143–153, 1994

Metcalf D: The molecular control of cell division, differentiation commitment and maturation in haemopoietic cells. *Nature* 339:27–30, 1989

Murphy M, Reid K, Ford M, Furness JB, Bartlett PF: FGF2 regulates proliferation of neural crest cells, with subsequent neuronal differentiation regulated by LIF or related factors. *Development* 120:3519–3528, 1994

Murphy M, Drago J, Bartlett PF: Fibroblast growth factor stimulates the proliferation and differentiation of neural precursor cells *in vitro*. *J Neurosci Res* 25:463–475, 1990

Murphy M, Reid K, Brown MA, Bartlett PF: Involvement of leukemia inhibitory factor and nerve growth factor in the development of dorsal root ganglion neurons. *Development* 117:1173–1182, 1993

Murphy M, Reid K, Hilton DJ, Bartlett PF: Generation of sensory neurons is stimulated by leukemia inhibitory factor. *Proc Natl Acad Sci USA* 88:3498–3501, 1991

Murphy M, Reid K, Williams DE, Lyman SD, Bartlett PF: Steel factor is required for maintenance, but not differentiation, of melanocyte precursors in the neural crest. *Dev Biol* 153:396–401, 1992

Nordeen EJ, Nordeen KW: Neurogenesis and sensitive periods in avian song learning. *Trends Neurosci* 13:31–36, 1990

Nurcombe V, Ford MD, Wildschut JA, Bartlett PF: Developmental regulation of neural response to FGF-1 and FGF-2 by heparan sulfate proteoglycan. *Science* 260:103–106, 1993

Patterson PH: Environmental determination of autonomic neurotransmitter functions. *Annu Rev Neurosci* 1:1–17, 1978

Pownall ME: More to patterning than Sonic hedgehog. *Bioessays* 16:381–383, 1994

Ray J, Gage FH: Spinal cord neuroblasts proliferate in response to basic fibroblast growth factor. *J Neurosci* 14:3548–3564, 1994

Reid K, Nishikawa S-I, Bartlett PF, Murphy M: Steel factor directs melanocyte development *in vitro* through selective regulation of the number of c-kit$^+$ progenitors. *Dev Biol* 169:568–579, 1995

Reid K, Turnley AM, Maxwell GD, Kurihara Y, Kurihara H, Bartlett PF, Murphy M: Multiple roles for endothelin in melanocyte development: regulation of progenitor number and stimulation of differentiation. *Development* 122:3911–3919, 1996

Reynolds BA, Tetzlaff W, Weiss S: A multipotent EGF-responsive striatal embryonic progenitor cell produces neurons and astrocytes. *J Neurosci* 12:4565–4574, 1992

Reynolds BA, Weiss S: Generation of neurons and astrocytes from isolated cells of the adult mammalian central nervous system. *Science* 255:1707–1710, 1992

Richards LJ, Kilpartrick TJ, Dutton R, Tan S-S, Gearing DP, Bartlett PF, Murphy M: Leukemia inhibitory factor or related factors promote the differentiation of neuronal and astrocytic precursors within the developing murine spinal cord. *Eur J Neurosci* 8:291–299, 1996

Richards LJ, Kilpatrick TJ, Bartlett PF: *De novo* generation of neuronal cells from the adult mouse brain. *Proc Natl Acad Sci USA* 89:8591–8595, 1992a

Richards LJ, Kilpatrick TJ, Bartlett PF, Murphy M: Leukemia inhibitory factor promotes the neuronal development of spinal cord precursors from the neural tube. *J Neurosci Res* 33:476–484, 1992b

Richards LJ, Murphy M, Dutton R, Kilpatrick TJ, Puche AC, Key B, Tan SS, Talman PS, Bartlett PF: Lineage specification of neuronal precursors in the mouse spinal cord. *Proc Natl Acad Sci USA* 92:10079–10083, 1995

Sanes JR: Lineage tracing. The latest in lineage. *Curr Biol* 4:1162–1164, 1994

Shah NM, Groves AK, Anderson DJ: Alternative neural crest cell fates are instructively promoted by tgf-beta superfamily members. *Cell* 85:331–343, 1996

Shah NM, Marchionni MA, Isaacs I, Stroobant P, Anderson DJ: Glial growth factor restricts mammalian neural crest stem cells to a glial fate. *Cell* 77:349–360, 1994

Sieber-Blum M, Cohen AM: Clonal analysis of quail neural crest cells: they are pluripotent and differentiate *in vitro* in the absence of noncrest cells. *Dev Biol* 80:96–106, 1980

Snyder EY, Taylor RM, Wolfe JH: Neural progenitor cell engraftment corrects lysosomal storage throughout the MPS VII mouse brain. *Nature* 374:367–370, 1995

Stewart CL, Kaspar P, Brunet LJ, Bhatt H, Gadi I, Kontgen F, Abbondanzo SJ: Blastocyst implantation depends on maternal expression of leukaemia inhibitory factor. *Nature* 359:76–79, 1992

Temple S: Division and differentiation of isolated CNS blast cells in microculture. *Nature* 340:471–473, 1989

Ware CB, Horowitz MC, Renshaw BR, Hunt JS, Liggitt D, Koblar SA, Gliniak BC, McKenna HJ, Papayannopoulou T, Thoma B, Cheng L, Donovan PJ, Peschon JJ, Bartlett PF, Willis CR, Wright BD, Carpenter M: Targeted disruption of the low-affinity leukemia inhibitory factor receptor gene causes placental, skeletal, neural and metabolic defects and results in perinatal death. *Development* 121: 1283–1299, 1995

Wetts R, Fraser SE: Microinjection of fluorescent tracers to study neural cell lineages. *Development* 2:1–8, 1991